Our Reference No. 9369-153

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Gijs van Rooijen, Richard Glenn Keon, Yin Shen and Joseph Boothe	)	
	)	
Serial No.: **09/643,755**	)	Group No.: 1638
	)	
Filed: **August 23, 2000**	)	Examiner: Georgia L. Helmer
	)	
For: **Commercial Production of Chymosin in Plants**	)	

Honorable Commissioner of Patents
Alexandria, Virginia 22313-1450
U.S.A.

Dear Sir:

DECLARATION UNDER 37 CFR §1.132

I, David T. Dennis, a citizen of Canada, and resident of Elginburg, Ontario, Canada, declare that the following facts are within my knowledge and are true.

1. I reside at RR#1, Elginburg, Ontario, Canada, K7L 3N6.

2. I am currently President and CEO of Performance Plants, Kingston, Ontario, Canada.

3. I have been working in the area of plant biotechnology since 1984 and as a plant biochemist since 1963. My curriculum vitae is attached to this Declaration as Exhibit A.

4. I have read and understood the disclosure and claims of U.S. Patent Application No. 09/643,755 filed August 23, 2000 (hereafter "the Application").

5. I have read and understood the office action that issued on the Application on December 16, 2003. The Examiner is of the view that the claims are not novel and are obvious in view of Patent Cooperation Treaty application WO 92/01042 to Willmitzer et al. (hereinafter "Willmitzer").

6. I have read and understood the claims that are attached to this Declaration as Exhibit B that I understand that Applicant is filing in response to the office action dated December 16, 2003. My comments below are based on the amended claims in Exhibit B (hereinafter "the amended claims").

7. The Applicant has developed a method 1) to express chymosin at high levels (i.e. in excess of 0.5%) in seed and 2) to isolate the chymosin from the seed. I am of the opinion that the expression of chymosin in seed at level in excess 0.5% is a significant advance over Willmitzer even without further limitation of the purification method.

8. The purification of a recombinant protein expressed in plants is necessary for the protein to be of any commercial value. To achieve this, the protein must initially be present at a high percentage of the extractable tissue protein and be in a tissue that does not denature the protein during extraction.

9. Willimitzer teaches that chymosin can be produced in tobacco leaves. The chymosin was detected by Western blots and by a new protease activity in the extracts. The concentration of 0.1-0.5% is only estimated from the Western Blot. Willmitzer also teaches that chymosin can be expressed in potato tubers but there is no description of the characterization of the chymosin protein. There

is no evidence presented that shows the integrity of the chymosin protein and no attempt is made to purify the protein to homogeneity.

10. The use of leaves and tubers would not be the preferred site to express a protein such as chymosin. The concentrations of proteins in leaves are low and subjected to denaturing conditions such as polyphenols, proteases and acid conditions. Potato tubers have similar problems. The expression of a protein in a seed is much more desirable as it produces active unmodified protein.

11. The purification of a protein from plant tissue to homogeneity is not a simple or routine task. It is something that has to be developed for each protein that is isolated. Willmitzer lists the types of protein purification methods generally available but there is no evidence that they would be successful when applied to chymosin, particularly when attempting to isolate chymosin from seed. The purification steps recited in the amended claims do specifically address this issue and are novel compared with Willmitzer.

12. The methodologies of protein purification have to be developed and established for each protein that is purified. This is especially true for the purification of chymosin from plant seeds where pure active protein is required in large amounts. Applicant has developed a methodology that permits purification of chymosin from plant seeds. Applicant has achieved purification of chymosin from plant seed by a methodology which involves the steps of fractionating crushed seed into an oil fraction, an aqueous fraction and a fraction comprising insoluble material and then subsequently contacting the aqueous fraction containing the chymosin with a protein binding resin. These method steps are not obvious in light of Willmitzer.

13. In summary, I believe that Applicant is entitled to claim a method for the expression and isolation of chymosin from seeds as specified in the amended claims. I am of the opinion that the amended claims are novel and not obvious in view of Willmitzer.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Application or patent resulting therefrom.

26 May 2004
Date

D. T. Dennis
David T. Dennis